# City of Milwaukee Health Department
# Disease Control and Prevention
# 1994-2004 *Cryptosporidium* and *Giardia*
# Surface Water Monitoring Project Summary

## History

The City began investigating the presence of *Cryptosporidium* and *Giardia* in surface waters around Milwaukee in 1994 in order to better understand the occurrence and distribution of these pathogens in the water, and to better define potential sources of *Cryptosporidium* and *Giardia* in waters that flow into Lake Michigan, because Lake Michigan is used as a water source for the City of Milwaukee. Lake Michigan water is treated at two Purification Plants in the City of Milwaukee; Linnwood and Howard. The intakes for the water Purification Plants are located several miles from shore, to avoid the influences of direct storm water run-off and the Milwaukee River Watershed, which itself contains run-off and storm water, and dilute sewage during combined sewer overflows, sanitary sewer overflows and sewage blending episodes.

## Evolution of the Watershed Monitoring Project

As the early objectives of characterizing occurrence and distribution of *Cryptosporidium* and *Giardia* in the Milwaukee River Watershed were met, monitoring was focused to answer more specific and relevant questions. From a drinking water perspective, it is important to know how much *Cryptosporidium* is in the Watershed as it enters Lake Michigan, since *Cryptosporidum* is not affected by chlorination routinely used in drinking water disinfection (though *Cryptosporidium* are significantly removed by filtration and ozonation). Since the Watershed represents a large potential source of pollution to Lake Michigan, it is important to monitor water quality indicators like *E. coli*, and to watch for upward trends and spikes in occurrence of *Cryptosporidium*.
In the early years of the Milwaukee Health Department (MHD) Watershed Monitoring Project, the MHD tracked *E. coli* levels in samples analyzed for *Cryptosporidum* and *Giardia* to evaluate use of *E. coli* as an indicator for these pathogens. The MHD learned that, although the presence of *E. coli* does indicate fecal contamination is present, it does not predict the presence of *Cryptosporidium* or *Giardia* because of differences in sources of *E. coli* and *Cryptosporidium* or *Giardia*, the ability of *E. coli* to increase in numbers in a water sample, and the insensitivity of laboratory methods used to detect *Cryptosporidium* and *Giardia*. Thus although *E. coli* is a useful indicator of pollution and water quality, it cannot be used to predict the occurrence of *Cryptosporidium* or *Giardia*.
Other changes that occurred in the monitoring scheme were:

- Elimination of “Rain Event” monitoring, since *Cryptosporidium* and *Giardia* occur both in wet and dry weather, and the MHD did not have access to technology to collect first flush samples, or have a reason to acquire that technology.

- Elimination of rural and upstream sampling sites, since a large enough data base had been developed and showed that Cryptosporidium and Giardia occurred both upsatream and down, in samples from each of the three rivers (Milwaukee, Menomonee and Kinnickinnic.
- Elimination of individual sampling sites from urban areas along the Milwaukee, Menomonee and Kinnickinnic Rivers except for the Milwaukee River site, because monitoring there serves a dual purpose (monitor individual river contribution of *E. coli* and recreational water quality).
- Monitoring the River Confluence. In monitoring the Confluence, it is possible to monitor the combined waters of the three rivers before they flow into Lake Michigan.
- Obtaining information about the genotype of as many of the *Cryptosporidium* genotypes as is possible (not all geneotypes are harmful to humans)
- Obtaining information about morphology of *Cryptosporidium* and *Giardia* that is detected

## A few words about *Giardia* in Watershed samples

Though the City tests each sample for *Cryptosporidium* and *Giardia*, and presence of *Giardia* provides useful information about potential sources of fecal contamination, presence of *Giardia* in Watershed samples is less of a concern because:

- *Giardia* is susceptible to chlorination
- *Giardia* is more susceptible to ozonation than *Cryptosporidium*
- Like Cryptosporidium, *Giardia* is significantly reduced by filtration

The City has chosen to focus efforts on *Cryptosporidium*, because a recent scientific literature review confirmed that Cryptosporidium should remain our benchmark pathogen when considering conditions necessary to assure the removal of *Cryptosporium.* It is, however, important to periodically check that *Giardia* is not occurring more frequently or in higher concentrations than has been observed. Because of this fact, most of the data shown will describe the challenge of *Cryptosporidium* that needs to be addressed by water purification.

## Recreational Water Quality

The Milwaukee River is used for recreational purposes, such as rowing and Riversplash. Data from Watershed monitoring can assist in estimating risk due to limited contact with river water, though, since river water quality is variable, the best advice to the public is to assume there is contamination from a number of sources (as with all natural waters) and practice good personal hygiene.

South Shore and Bradford beaches are used for swimming (potential full-body contact), and ingestion of water that might contain *Cryptosporidium* or *Giardia* is possible. It is important to know if concentrations of *Cryptosporidium* or *Giardia* might be present that have the potential to cause disease in healthy humans (Immunocompromised individuals should avoid activities that might result in ingestion of unpurified water).

# *Cryptosporidium-Giardia* Surface Water Monitoring Activities in 2004

**Overview of MHD Surface Water Monitoring Activities: The following surface water quality monitoring was conducted by the MHD in 2004 and in previous years:**

- Watershed Monitoring
    1. *Cryptosporidium* and *Giardia* monitoring
    2. Riversplash *E. coli* monitoring, flushing tunnel operation information
    3. River (*E. coli* and Dissolved Oxygen) data from Milwaukee Metropolitan Sewerage District (MMSD)

- Recreational Water Quality (Beaches)
    1. *E. coli*
    2. *Cryptosporidium* and *Giardia*

- Waste Water Treatment Plant (WWTP) effluent data
    1. *E. coli* during beach season
    2. *Cryptosporidium* and *Giardia* during sewage blending events

- Storm water data from MMSD
    1. Review of *E. coli* results
    2. Milwaukee Water Water Works (MWW) "Raw" (Lake Michigan) or "Intake," untreated water, for numerous parameters that MWW monitors

**The following data (from activities listed above) will be presented in this 2004 *Cryptosporidium/Giardia* Monitoring Report:**

- *Cryptosporidium* and *Giardia* data from Confluence and Milwaukee River upstream sites, Beaches and WWTP
    1. Monthly samples from the River Confluence
    2. Periodic samples from Milwaukee River
    3. Twice-monthly samples from Bradford and South Shore Beaches
    4. Sewage blending Event samples from JI-WWTP
- *E. coli* data from sites monitored for *Cryptosporidium* and *Giardia*
- *Cryptosporidium* and *Giardia* in samples from MWW untreated and plant effluent water 1994-2004
    1. Twice-monthly sampling

# Results

## Table 1. Results from 2004 Monitoring

| **Date (Lab)** | **Milwaukee @ Erie and Polk (E) or Pleasant (P), Canoe Launch (CL) or South Shore Beach (SS), Bradford (BD) or JI-WWTP Blending Event Effluent (JIWWTPD)** | | **River Confluence** | | **Rainfall, Inches, Mitchell Field, within 24 hours prior to sampling** |
|---|---|---|---|---|---|
| | Crypto Oocysts/L | ***Giardia*** Cysts/L | Crypto Oocysts/L | ***Giardia*** Cysts/L | |
| 03/11/04 (WSLH) | <0.2 (E) | 0.4 (E); 50% DAPI+, 50% DAPI-, amorphous | <0.14 | 0.27; 100% DAPI + | 0.011 |
| 03/26/04 (MHD) | <0.15 (JI WWTP) | 8.8 (JI WWTP) | - | - | 0.71 |
| 04/14/04 (WSLH) | 0.14 (E) DAPI+, 4 nuclei, typical internal structure | <0.14 | <0.13 | <0.13 | 0.00 |
| 05/14/04 (MHD) | <0.14 (JIWWTP) | 117.76 (JIWWTP) | - | - | 0.45 |
| 05/18/04 (WSLH) | <0.14 (SS) | 0.40 (SS) DAPI+, typical internal structure | 0.28 DAPI+, typical internal structure | 0.28 DAPI +, typical internal structure | 0.59 |
| 06/09/2004 (WSLH) | <0.14 (SS) | <0.14 (SS) | - | - | - |
| 06/09/2004 (WSLH) | <0.13 (BD) | <0.13 (BD) | - | - | |
| 06/23/2004 (WSLH) | <0.16 (SS) | 0.64 (SS) DAPI+ Internal structure typical | <0.13 | 0.13 DAPI+ | - |
| 06/23/2004 | <0.15 (BD) | <0.15 (BD) | - | - | |
| 07/14/2004 | <0.14 (SS) | 0.14 (SS) Empty oocyst | - | - | - |
| 07/14/2004 | <0.24 (BD) | 3.53 (BD) All amorphous | - | - | |
| 07/21/2004 | <0.125 (SS) | 0.625 (SS) DAPI - | <0.14 | 0.14 DAPI - | - |
| 08/18/2004 | <0.15 (SS) | 0.15 (SS) | - | - | 0.05 |
| 08/18/2004 | <0.11 (BD) | <0.11 (BD) | - | - | |
| 08/25/2004 | - | - | <0.14 | <0.14 | 0.53 |
| 09/08/2004 | <0.1 (SS) | 1 (SS) 10% DAPI+,internal struct | <0.125 | 0.38 DAPI- | - |
| 10/13/04 | <0.15 (E) | 0.15 (E) | <0.1 | 0.10 DAPI- | - |

*"DAPI+" means that the organism isolated in EPA Method 1623 has nuclear material that stained with DAPI dye, and confirms the presence of Crypto or Giardia detected by Immunofluorescence. DAPI+ staining means the isolated organism may be viable. DAPI- organisms may be dead, resistant to DAPI staining, or other organisms.*

**Figure 1. Frequency of Detection of Cryptosporidium and Giardia in Watershed or Near-Shore Areas 2001-2004**

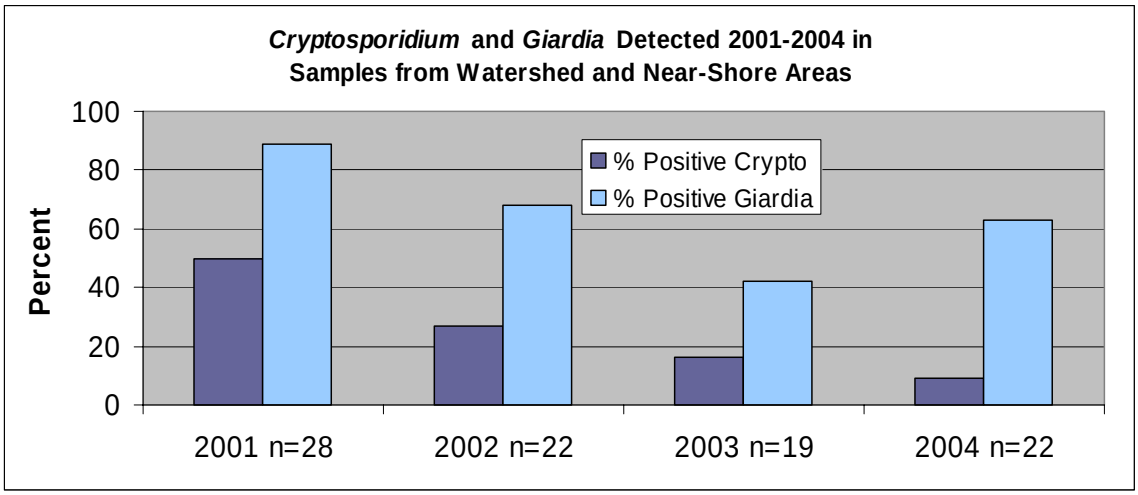


**Figure 2. Frequency of Detection of *Cryptosporidium* in Watershed and Near-Shore Areas (by Location) 1994-2004**

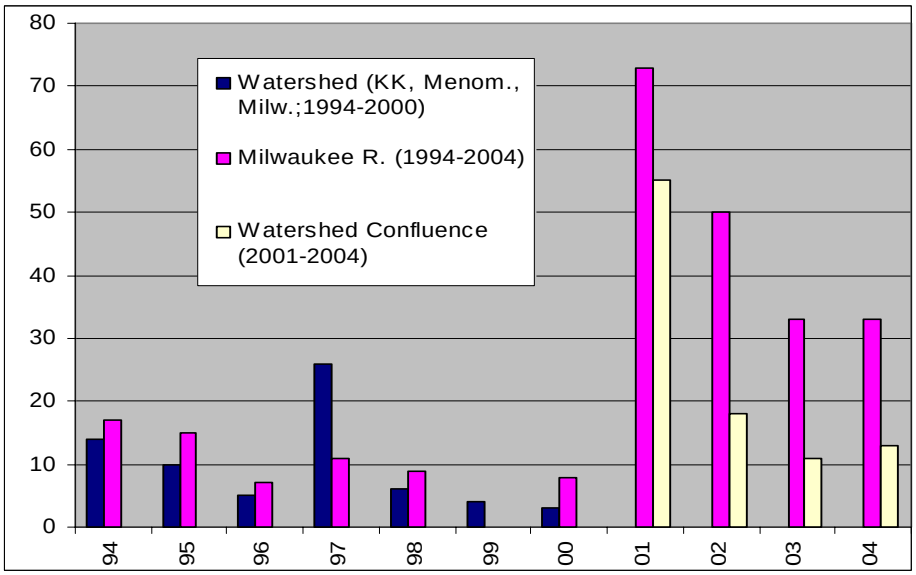

**Figure 3. Frequency of Detection of *Giardia* in Watershed and Near-Shore Areas (by Location) 1994-2004**

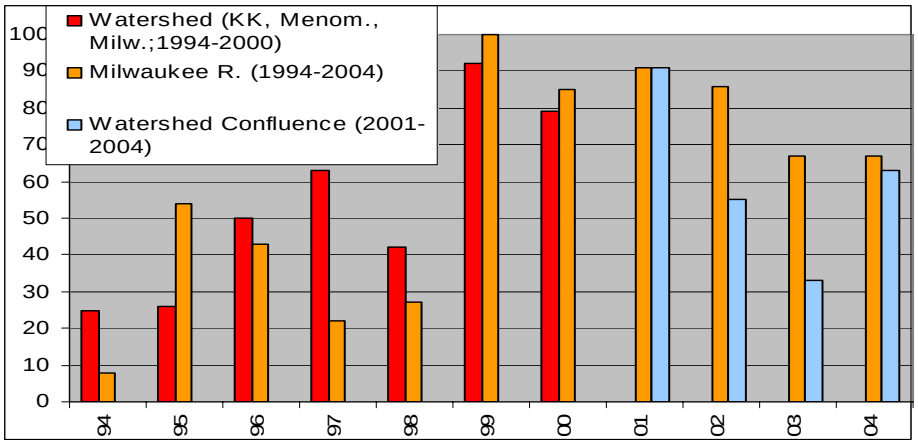


**Figure 4. *Cryptosporidium* Concentrations (Oocysts/L) in Waste Water Samples**

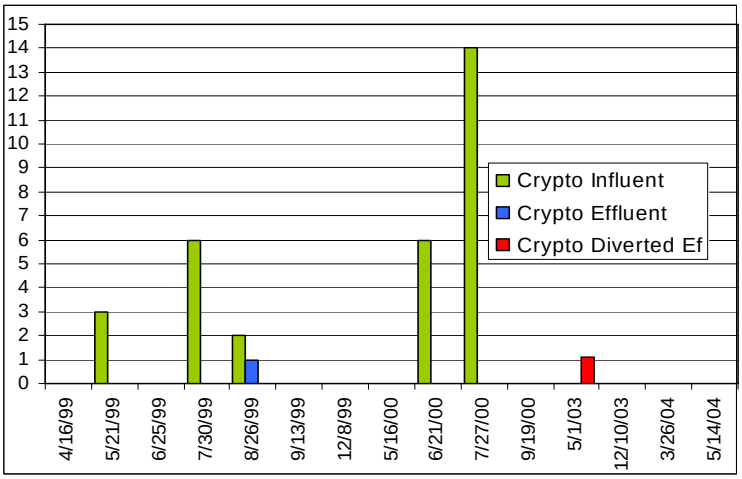


**Figure 5. *Giardia* Concentrations (Cysts/L) in waste Water Samples**

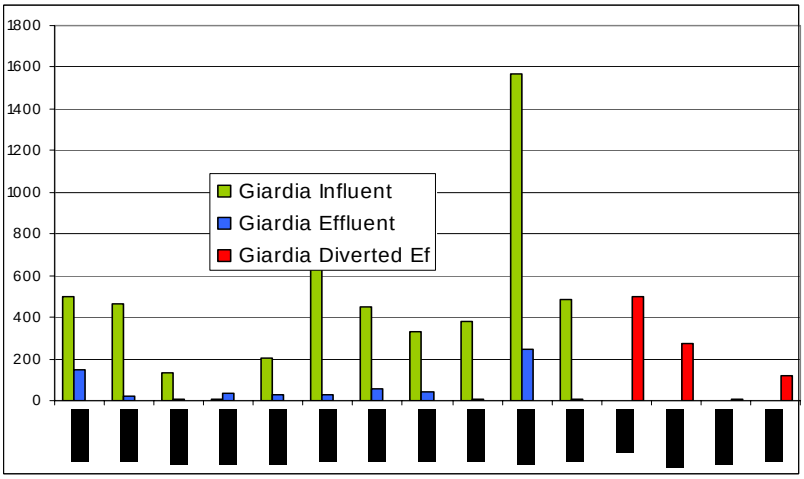

**Table 2. Ranges of *Cryptosporidium* and *Giardia* Concentrations (When Detected) in Samples from MWW Purification Plants (Raw, Untreated water) and other Locations**

| | **Linn/How Intake (Lake Michigan, 1995-2004)** | **River Confluence 1994-2004 (n=44)** | **Watershed 1994-2004 (n=287)** | **JI WWTP Effluent (1999-2000, n=11)** | **JI WWTP Blended Effluent (2003-2004, n=4)** | **JI WWTP Influent (1999-2000, n=11)** |
|---|---|---|---|---|---|---|
| *Cryptosporidium* (oocysts/L) | 0.006-0.251 | 0.1-0.3 | 0.1-4.1 | 1 (1 sample positive) | 1.1 (1 sample positive) | 2-14 |
| *Giardia* (cysts/L) | 0.010-2.6 | 0.1-7.0 | 0.1-179 | 6-248 | 8.8-500 | 6-1571 |

**Table 3. *Cryptosporidium*: Possible Environmental Sources**

| **Potential Environmental Source** | **Number of Samples analyzed (N=)** | **Percent of Samples Positive** | **Range Among Detects oocysts/L** | **Average Among Detects oocysts/L** |
|---|---|---|---|---|
| Agricultural: Milw R North (MHD/DNR) & Cedar Ck (USGS) | N=48 | 31 | 0.34-5.9 | 1 |
| Upstream WWTP (USGS) | N=64 | 28 | 0.4-4.65 | 1.67 |
| JI WWTP Effluent (MHD 1994-2000) | N=69 | 12 | 0.8-33 | 6.64 |
| CSOs (MHD,USGS) | N=3 | 67 | 16-33 | **Not Determined** |
| JI Blended Effluent, 2003 (MHD) | N=2 | 50 | 1.1 (1 sample positive) | **Not Determined** |
| **JI WWTP Effluent (1999-2000)** | N=11 | 9 | 1 (1 sample positive) | **Not Determined** |

## Table 4. *Cryptosporidium*: Genotype Information

| Genus detected | Species detected | Number of times detected (% of total not genotyped) | Number of times detected (% of total genotyped) | genotype | Pathogenic to "healthy" adults?/ID50/ref | Pathogenic to immunocompromised Humans?/ID50 |
|---|---|---|---|---|---|---|
| *Cryptosporidium* | *parvum* | **?+** | 1 (4) | Human (Genotype 1") | Yes, min dose=30? (DuPont, 1995) | Yes/? |
| *Cryptosporidium* | *parvum* | | - | Bovine (Genotype 2) | Yes, min dose=30? (DuPont, 1995), 10 oocysts, TAMU genotype (Okhuysen, 1999) | Yes/? |
| *Cryptosporidium* | *baileyi* | **?+** | 1 (4) | - | No/-/Xiao, CDC | Yes/? |
| *Cryptosporidium* | *muris* | **?+** | - | - | ? | Yes (epa ref)/? |
| *Cryptosporidium* | ???, reactive with antibodies used 1993-2003, at least *muris*, *baileyi* (Dynabeads) | **?+** | - | - | ? | ? |
| *Cryptosporidium* | ??(possum?) | - | 1 (4) | - | No/-/Xiao, CDC | Yes/? |
| *Cryptosporidium* | *andersoni* | - | 3 (11) | - | No/-/ | ?/?Yes? |
| *Cryptosporidium* | mouse | - | - | - | ? | Yes/? |
| **Not Detected, but have infections in humans documented** | | | | | | |
| *Cryptosporidium* | *canis* | - | | - | Yes(Akiyoshi, others)? | Yes |
| *Cryptosporidium* | *felis* | - | | - | Yes (Akiyoshi, others)? | Yes |
| *Cryptosporidium* | *parvum*, human, water | - | | Water Genotype | Yes, min dose=30? (DuPont, 1995) | Yes/? |
| *Cryptosporidium* | *meleagridis* | - | | - | Yes (Akiyoshi, 2003) Similar to parvum | Yes/? |
| 27 samples Watershed and Near Shore, 22% positive, 3 C. andersoni (11% of total, 50% of detects); 1 C. baileyi (4% of total, 17% of detects); 1 C. ???(4% of total, 17% of detects); 1 *C. parvum*, human (4% of total, 17 % of detects) | | | | | | |

## Table 5. *E. coli* Averages (MPN/100 mL) by Location 2001-2004

| Canoe Launch or Pleasant St (n=) | Erie St (n=) | Watershed Confluence (n=) | South Shore Beach (n=) | Bradford Beach (n=) | JI WWTP Effluent (n=) | Blended Effluent (n=) |
|---|---|---|---|---|---|---|
| 237 | 91 | 85 | 510 | 415 | | 14,619 |

# Discussion

**Drinking Water**

Concentrations and frequency of *Cryptosporidium* and *Giardia* detected in samples from the Watershed were similar to those of previous years, indicating that these pathogens are not likely to present an increased challenge to removal or inactivation processes in use at the Water Purification Plants. The multiple barriers present should be adequate to protect the general public from these organisms in almost all cases. *Cryptospordium* and *Giardia* were each detected in less than 1 %, of finished water samples (n=412) analyzed 1996-2004, each several orders of magnitude below their respective infective doses (1, 2). There is no information that any *Cryptosporidium* or *Giardia* that were detected had the potential to infect humans since information about morphology or viability of any of these organisms is not available. Not all *Cryptosporidium* species or genotypes (3) are associated with infections in humans. There is also no information about the genotype of any of the rarely detected *Cryptosporidium* in finished water. As a precaution, any organism isolated is considered to be able to cause infection in humans.

The MHD and Waterworks are currently working to assess the realistic risk to those who drink tap water from the Linnwood or Howard Plants since there is only a theoretical chance that a healthy adult would consume an infective dose of *Cryptosporidium*.

**Recreational Water**

Only 6% Of all Recreational Water samples (n=36) were found to contain Cryptosporidium and 83% contained *Giardia*. Two samples (from South Shore and Bradford Beaches) contained greater than 2 *Giardia* cysts per liter (after adjustment for recovery, see Appendix), however, when one of the two positive samples was examined more closely, the *Giardia* were described as amorphous. Amorphous *Giardia* may not be able to establish infection in humans. Thus although concentrations of *Giardia* may sometimes be close to the infective dose (2), they may not represent organisms that can actually establish an infection. Sample sizes are too small to accurately describe the occurrence of *Cryptosporidium* and *Giardia* in swimming waters, or describe risk to bathers for illness from *Cryptosporidium* or *Giardia*, but the information can be used to document that *Cryptosporidium* and *Giardia* may be present in swimming water, and avoidance of swallowing water (per CDC guidance for all recreational waters) is advisable.

## References


1. DuPont, et al N. Engl. J. Med. 332:855-859 (1995)
2. USFDA Bad Bug Book http://www.cfsan.fda.gov/~mow/chap22.html
3. WI-DNR PUBL-WR420-95 *Cryptosporidium* ssp. Oocyst and *Giardia* Cyst Occurrence, Concentrations and Distributions in Wisconsin Waters, State of WI DNR (1995)
4. CDC, Healthy Swimming http://www.cdc.gov/healthyswimming/index.htm
5. Xiao, et al. J. Clin. Microbiol. 41(11): 5254-7 (2003)


## Appendix: Percent Recovery for Cryptosporidium and Giardia by Lab Analyzing and Methodology Used

| Time Period and Method | Percent Recovery | Notes |
|---|---|---|
| 1994 WI DNR Samples | >60 *Crypto*<br>>60 *Giardia* | 100 L filtered in field |
| 1994-1998 ICR IFA Method (MHD Labs) | 6.1-42.8, *Crypto*<br>24.5-51.6, *Giardia* | 100 L filtered in field |
| 1999-2004<br>EPA Method 1623 (MHD Labs, IMS/IFA) | 60-70, *Crypto*<br>50 *Giardia* | 10 L filtered in Lab |
| 2000-2001 PCR (selected from MHD samples, CDC) | Unknown, theoretically high sensitivity and specificity | 10 L filtered in Lab |
| April-September 2002 EPA Method 1623 (WSLH, flow cytometry and microscopy) | >60 *Crypto*<br>>60 *Giardia* | 10 L filtered in Lab |
| September 2002-December 2003 (WSLH, IMS/IFA) | >60 *Crypto*<br>>60 *Giardia* | 10 L filtered in Lab |
| 2003 Blended Jones Island Waste Water samples (WSLH, IMS/IFA) | >60 *Crypto*<br>>60 *Giardia* | 10 L filtered in Lab |